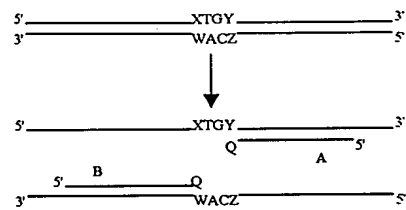
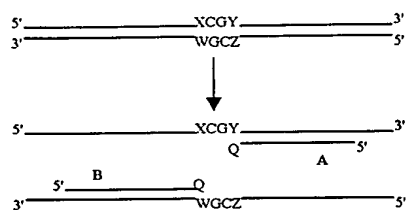


STEPS IN PCR/RE/LDR

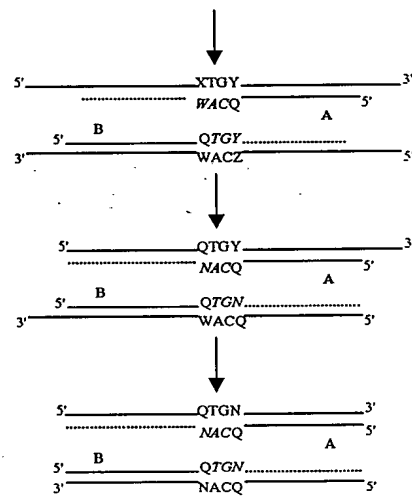
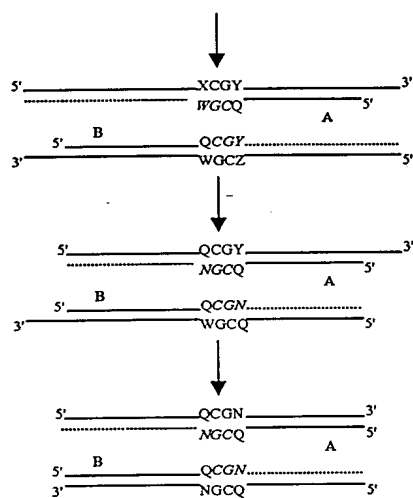
WILD-TYPE

MUTANT

1)



2)



3)

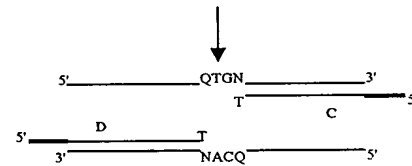
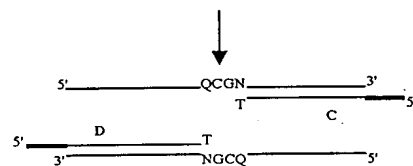
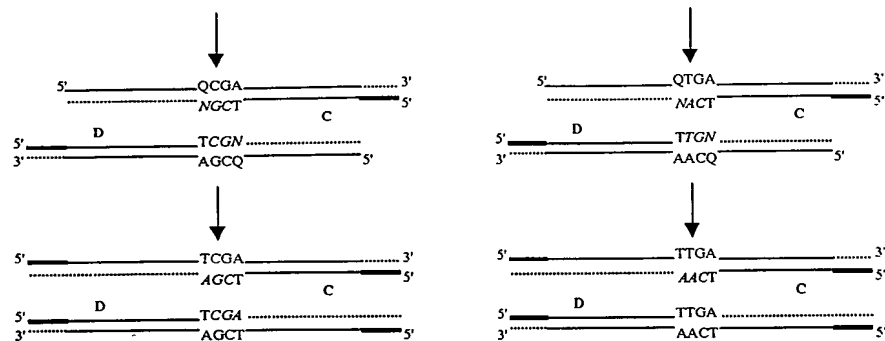


FIGURE 1

4)



5)

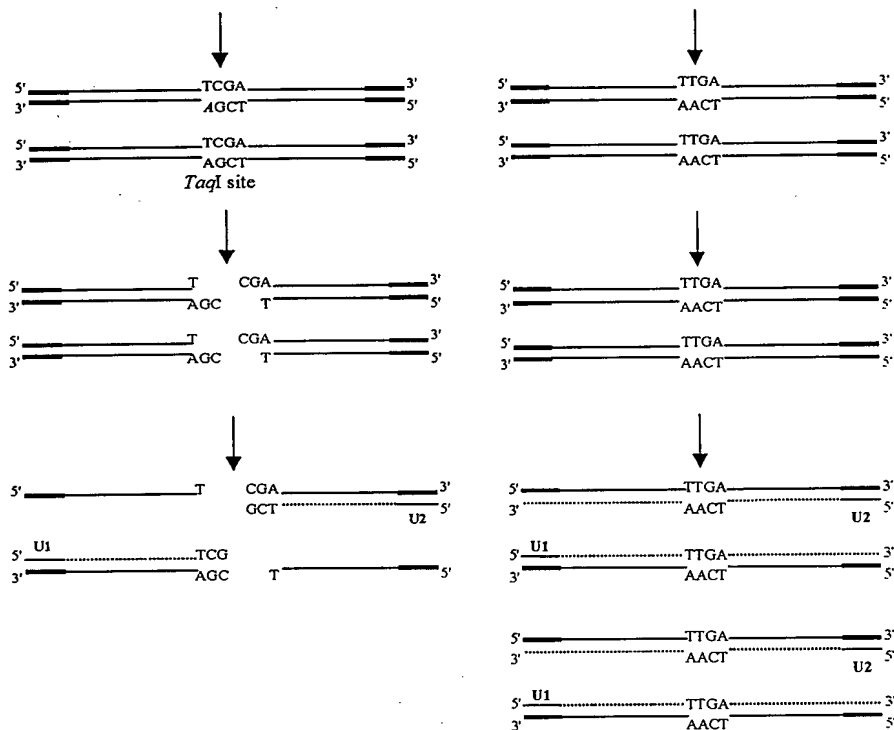
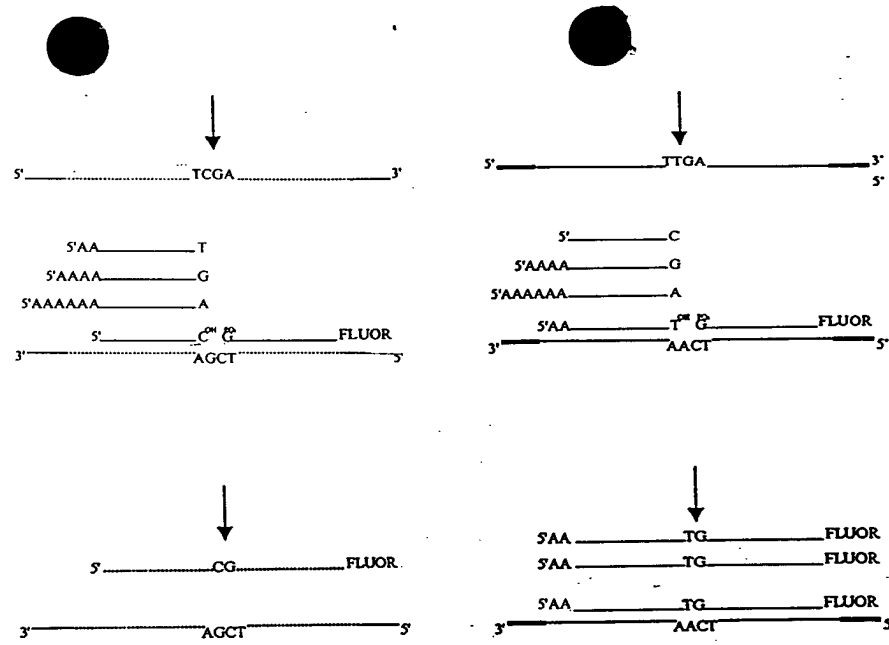


FIGURE 1 (Cont'd.)

7A)



7B)

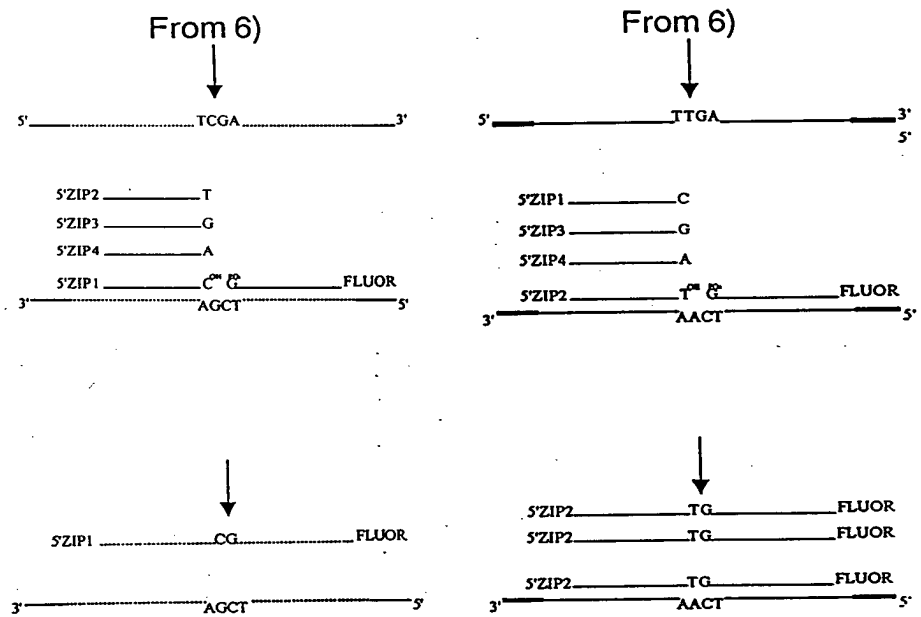


FIGURE 1 (Cont'd.)

004TED07032500

SAA ——— TG ——— FL

S CG FL

S'AAAAAA ——— A

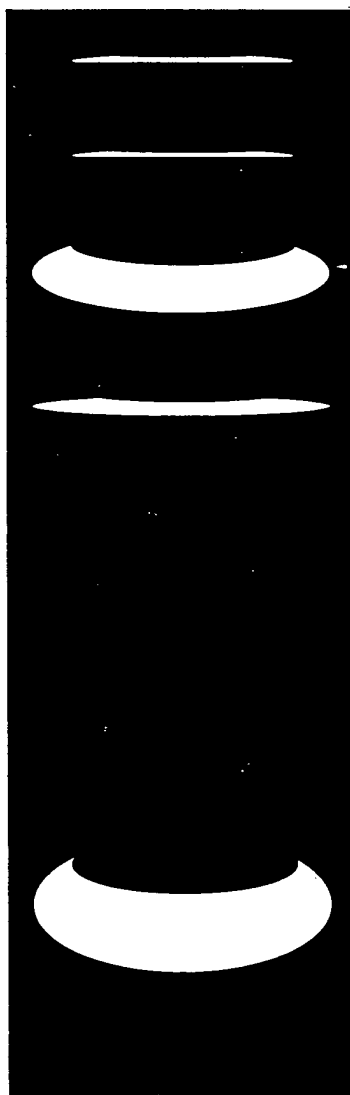
S'AAAA ——— G

S'G FL

S'AA ——— T

S' ——— C

I. GEL LANE



↑
INCREASING MASS

FIGURE 2

5'ZIP2 _____ TG _____ FLUOR


5'ZIP1 CG FLUOR

5'ZIP2 _____ T

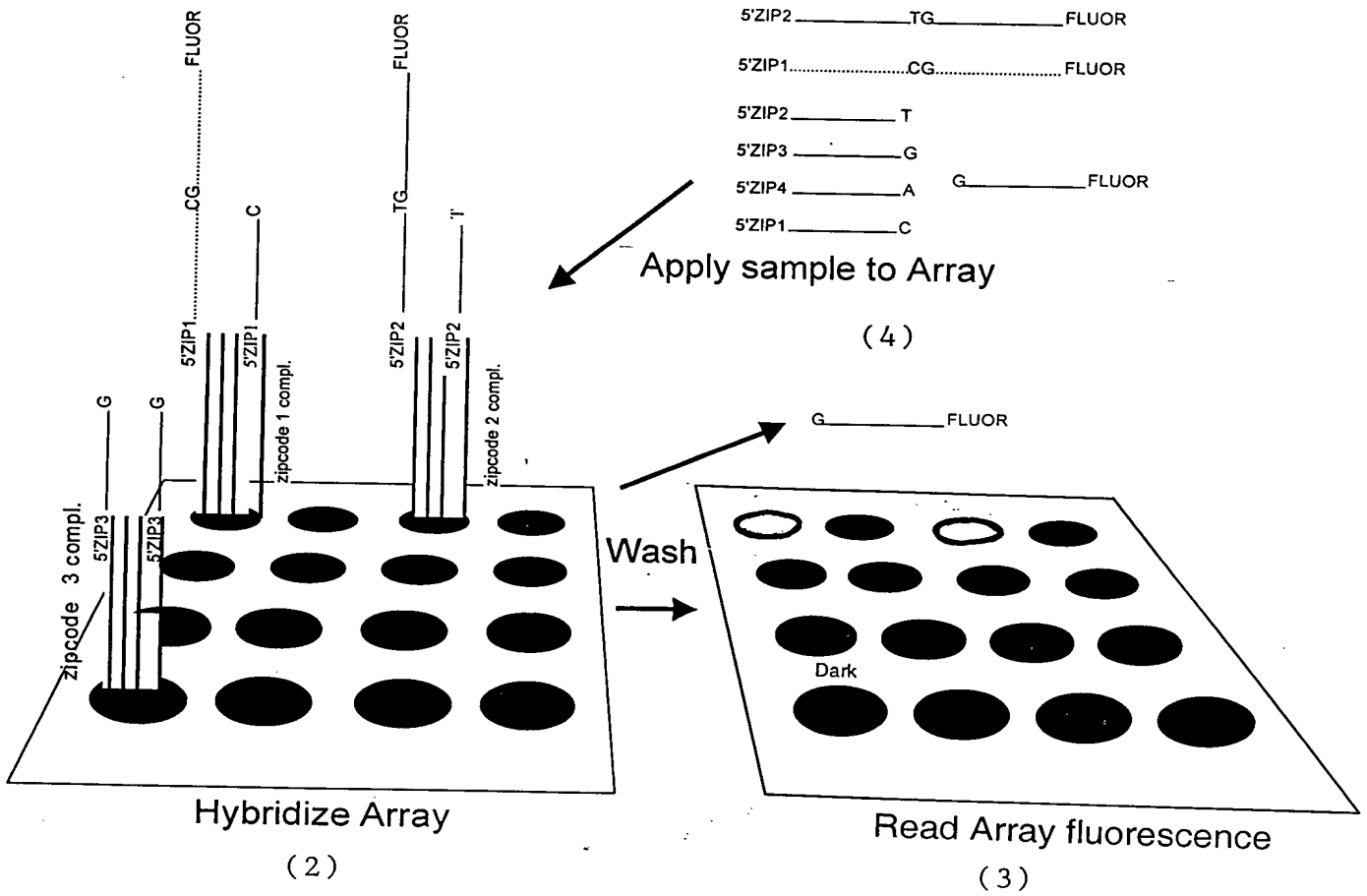
5'ZIP3 _____ G

5'ZIP4 _____ A G _____ FLUOR

5'ZIP1 _____ C

 Apply sample to Array

(4)



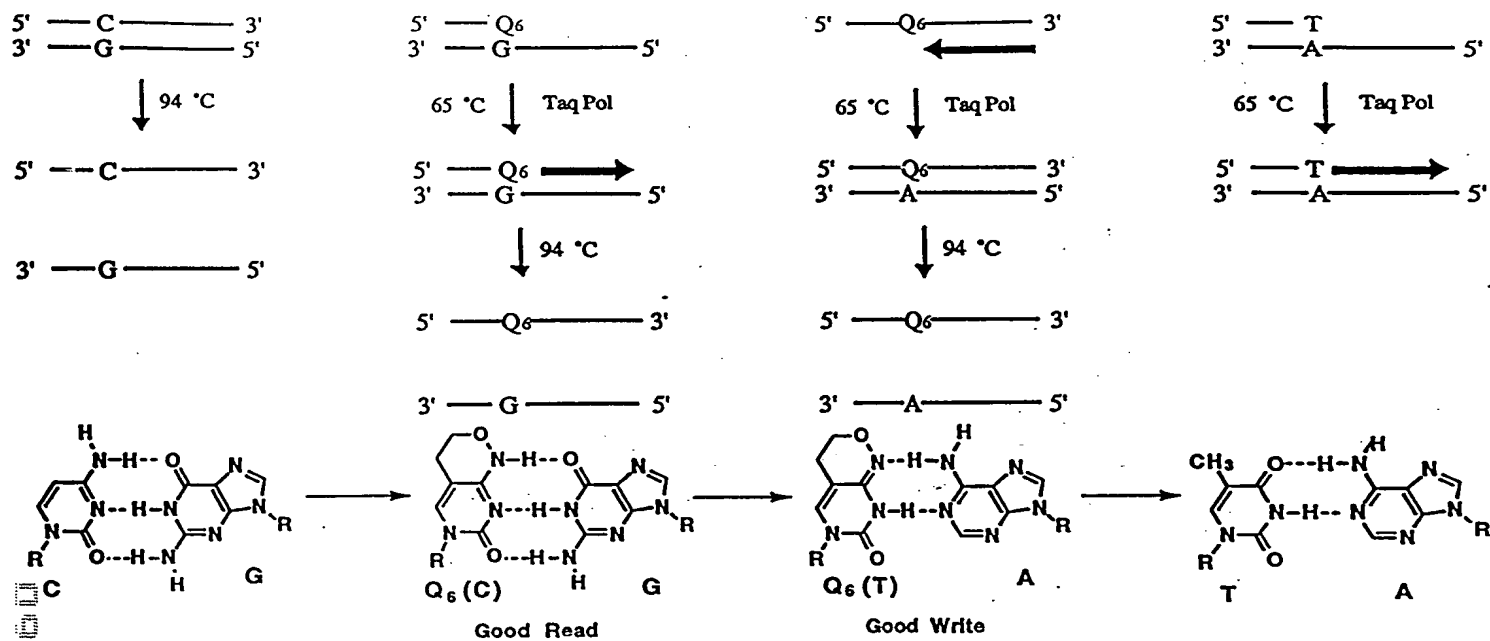


FIGURE 4

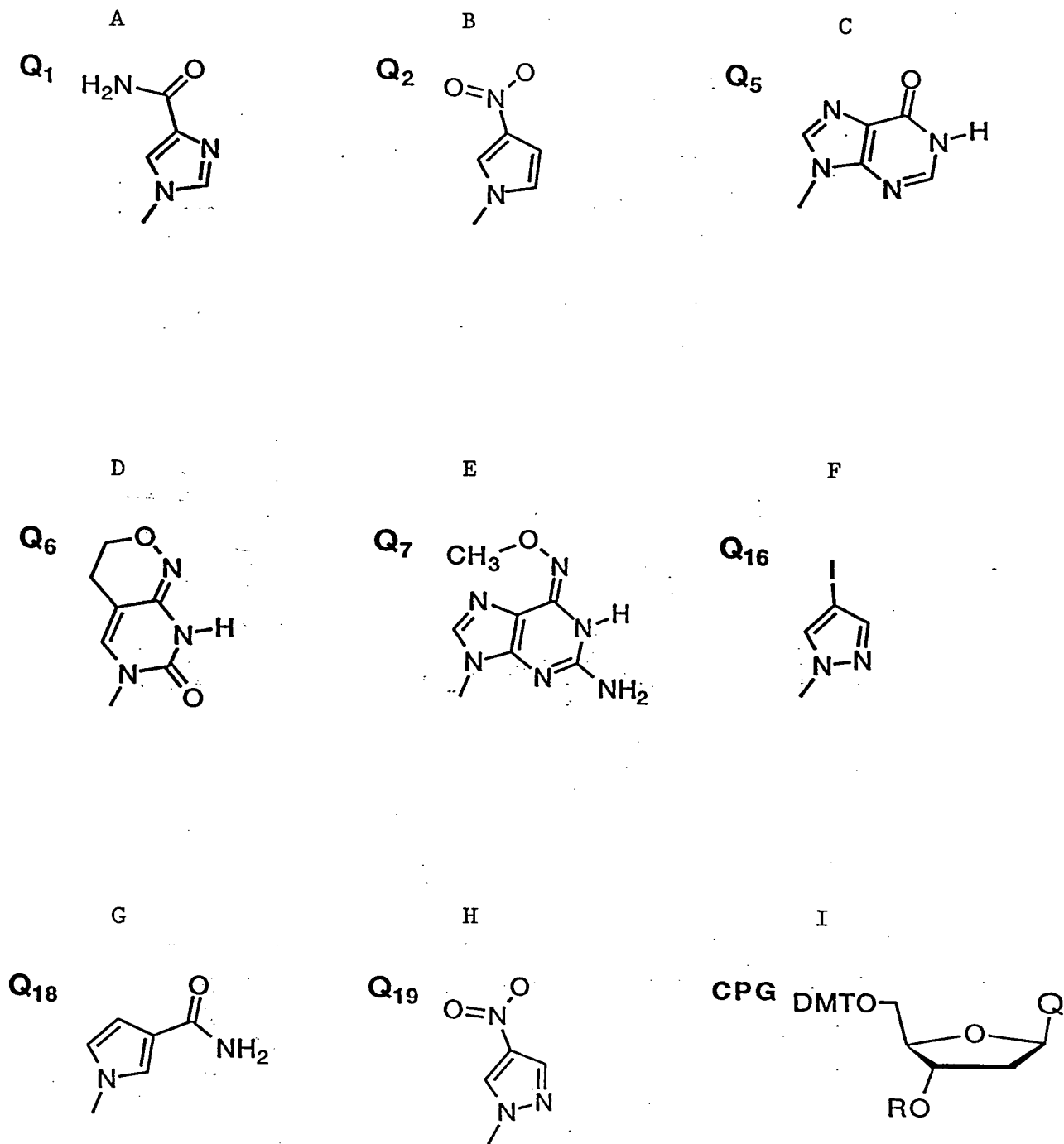


FIGURE 5

A

Primers

Ztop

p53zlp248

p53-248X

50 bp synthetic duplex DNA

p53-248XR

p53zlp248R

Zbot

codon 248



CTT GGA CGA GTT CAT ACG C

CTT GGA CGA GTT CAT ACG CGT TCC TGC ATG GGC GGC ATG A

5' TCT TCC TGC ATG GGC GGC ATG AAX → *pol*

|| ||| ||| ||| ||| ||| ||| |||

3' CA AGG AGC TAC CCG CCG TAC TTG GGC TCC GGG TAG GAG TGG TAG TAG TGT 5' (-)

5' GT TCC TGC ATG GGC GGC ATG AAC GGG AGG CCC ATC CTC ACC ATC ATC ACA 3' (+)

: ||| ||| ||| ||| ||| ||| ||| |||

pol-X TCC GGG TAG GAG TGG TAG TAG TCT T

C GGG TAG GAG TGG TAG TAG TCC ACC GCT GGG TCA AAC G

C ACC GCT GGG TCA AAC G

B

Primers

Ztop

p53zlp248T

p53-248Q_N

50 bp synthetic duplex DNA

p53-248Q_NR

p53zlp248TR

Zbot

codon 248



CTT GGA CGA GTT CAT ACG C

CTT GGA CGA GTT CAT ACG CGT TCC TGC ATG GGC GGC ATG AAT

5' TCT TCC TGC ATG GGC GGC ATG AAQ_N → *pol*

|| ||| ||| ||| ||| ||| ||| |||

3' CA AGG AGC TAC CCG CCG TAC TTG GGC TCC GGG TAG GAG TGG TAG TAG TGT 5' (-)

5' GT TCC TGC ATG GGC GGC ATG AAC GGG AGG CCC ATC CTC ACC ATC ATC ACA 3' (+)

: ||| ||| ||| ||| ||| ||| ||| |||

pol-Q_N TCC GGG TAG GAG TGG TAG TAG TCT T

T TCC GGG TAG GAG TGG TAG TAG TCC ACC GCT GGG TCA AAC G

C ACC GCT GGG TCA AAC G

C

LDR Primers

p53LDR248FCA

p53LDR248FCG

p53LDR248FCT

p53LDR248FCC

p53LDR248PGG

conversion products

Discrimination

Common

F-AAAAAA GC ATG GGC GGC ATG AAC A

F-AAAA GC ATG GGC GGC ATG AAC G

F-AA GC ATG GGC GGC ATG AAC T

F- GC ATG GGC GGC ATG AAC C

7-ligase

GG AGG CCC ATC CTC ACC ATC AT-block

3' (-strand)

... GTA TGC GCA AGG ACG TAC CCG CCG TAC TTG ACC TCC GGG TAG GAG TGG TAG TAG TGA ACC...

5'

FIGURE 6

002FE074B2500

A

Template	CCGG		CTGG		CGGG		CAGG		TCGA		GCGC		ACGT		CATG		CGCG	
Expctd prod	CCGG		CTGG		CGGG		CAGG		CCGG		CCGG		CCGG		CATG		CGCG	
primer 3' end	C	Q ₆	C	Q ₆	C	Q ₆	C	Q ₆	C	Q ₆	C	Q ₆	C	Q ₆	C	Q ₆	C	Q ₆
1st base	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
2nd base	C	C	T	T	G	G	A	A	G	C	G	G	G	G	A	A	G	G
minor 2nd										t			t	t	T	T		
base prod												c		c	c			
LANE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

CANG

CGNG

CTNG

CCNG

255

127

0

B

Template	CCGG		CTGG		CGGG		CAGG		TCGA		GCGC		ACGT		CATG		CGCG	
Expctd prod	T		T		T		T		T		T		T		T		T	
primer 3' end	T	Q ₆	T	Q ₆	T	Q ₆	T	Q ₆	T	Q ₆	T	Q ₆	T	Q ₆	T	Q ₆	T	Q ₆
1st base	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
2nd base	?	C	?	T	?	G	?	A	C	C	c	C	?	C	?	A	?	G
minor 2nd					g								?	?	c	?	g	g
base prod																		
LANE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

TANA

TGNA

TTNA

TCNA

255

127

0

FIGURE 7

A

Template	CCGG	CTGG	CGGG	CAGG	TCGA	GCGC	ACGT	CATG	CGCG
Exptd prod	GCGC	GTGC	GGGC	GAGC	GCGC	GCGC	GCGC	GATC	GGCC
primer 3' end	G Q ₅ Q ₇	G Q ₅ Q ₇	G Q ₅ Q ₇	G Q ₅ Q ₇	G Q ₅ Q ₇	G Q ₅ Q ₇	G Q ₅ Q ₇	G Q ₅ Q ₇	G Q ₅ Q ₇
1st base	G G G	G G G	G G G	G G G	G G G	G G G	G G G	G G G	G G G
2nd base	C C C	C C C	C C C	C C C	C C C	C C C	C C C	C C C	? ? ?
minor 2nd base prod		t t t	g g	A A a					
LANE	1 2 3	4 5 6	7 8	9 10 11 12	13 14 15	16 17 18	19 20 21	22 23 24	25 26 27

GANC

GGC

GTNC

GCNC



B

Template	CCGG	CTGG	CGGG	CAGG	TCGA	GCGC	ACGT	CATG	CGCG
Exptd prod	ACGT	ATGT	AGGT	AAGT	ACGT	ACGT	ACGT	AATT	AGCT
primer 3' end	A Q ₇ Q ₅	A Q ₇ Q ₅	A Q ₇ Q ₅	A Q ₇ Q ₅	A Q ₇ Q ₅	A Q ₇ Q ₅	A Q ₇ Q ₅	A Q ₇ Q ₅	A Q ₇ Q ₅
1st base	A A A	A A A	A A A	A A A	A A A	A A A	A A A	A A A	A A A
2nd base	C C C	C C C	C C C	C C C	C C C	C C C	C C C	C C C	c C c
minor 2nd base prod	?		?						
LANE	1 2 3	4 5 6	7 8	9 10 11 12	13 14 15	16 17 18	19 20 21	22 23 24	25 26 27

AANT

AGNT

ATNT

ACNT

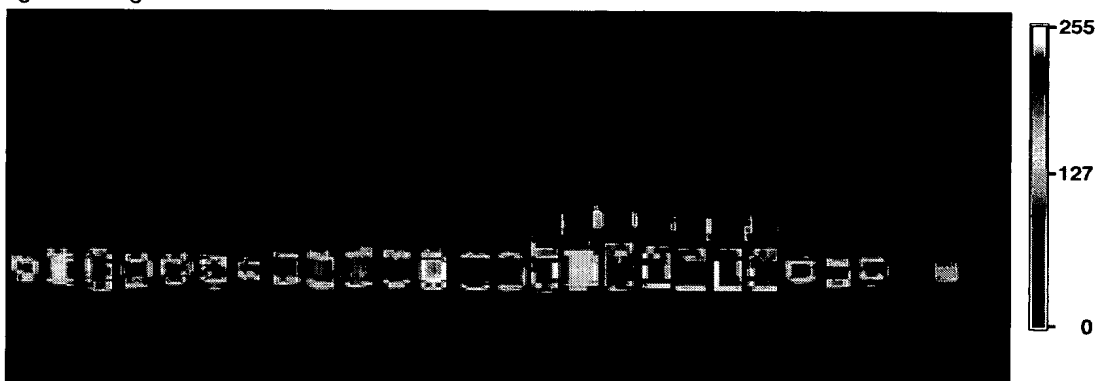


FIGURE 8

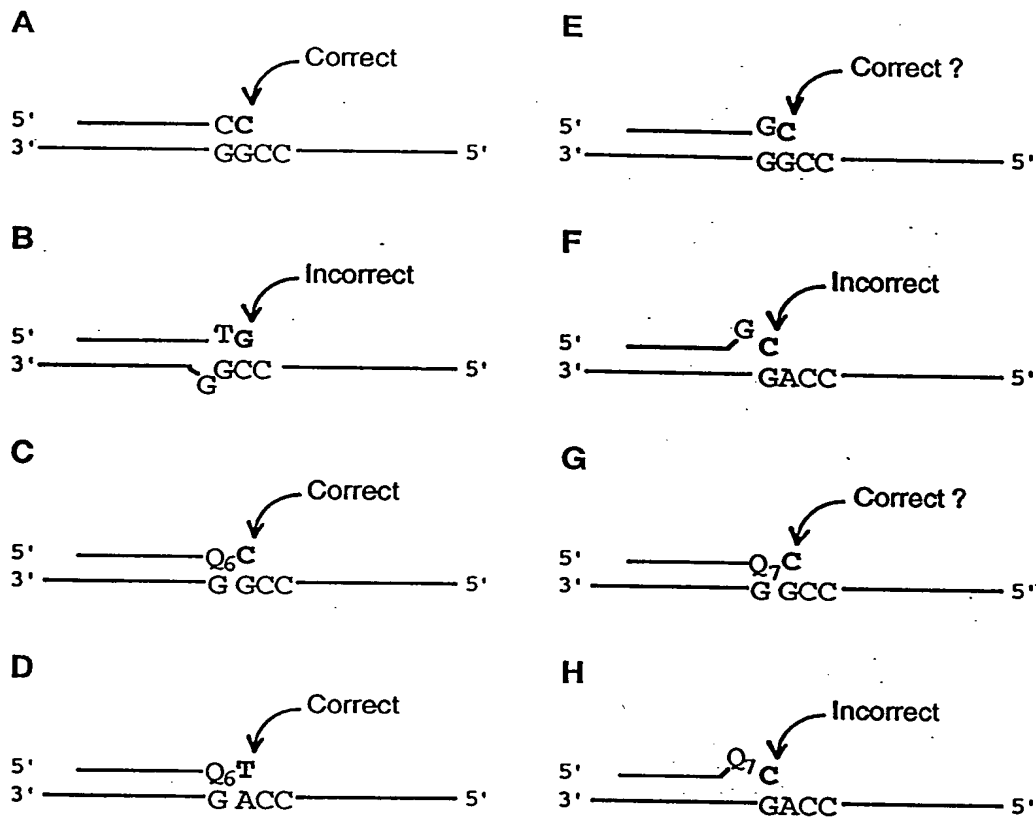
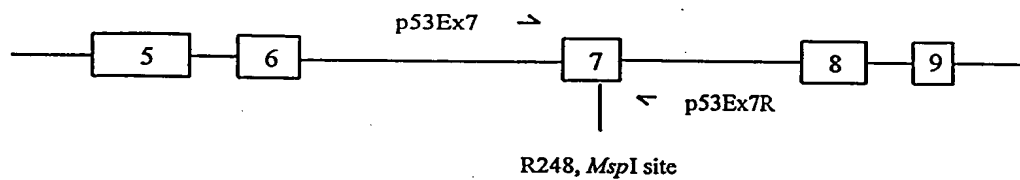
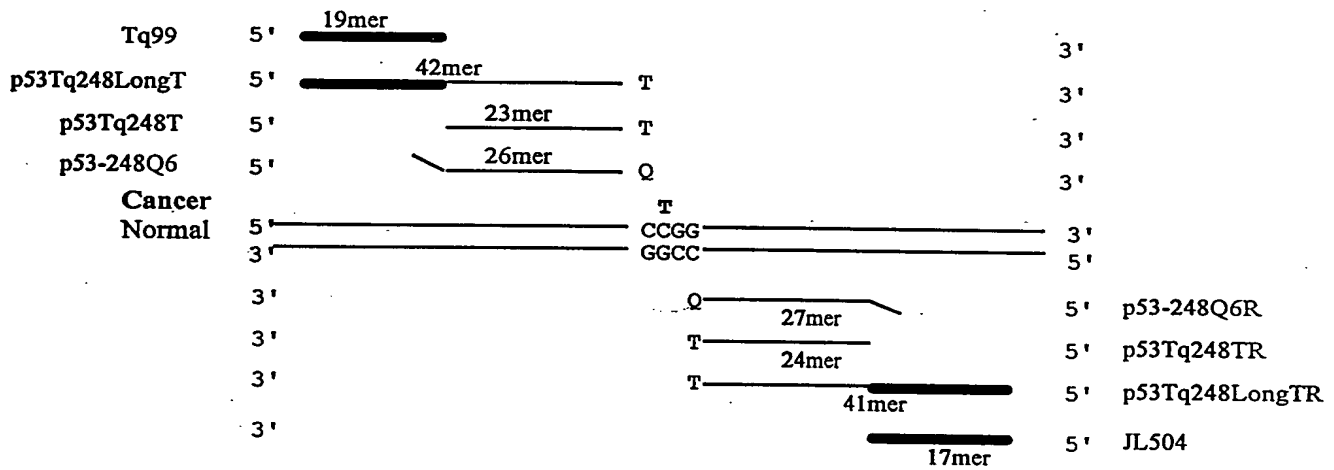


FIGURE 9

A



B



C

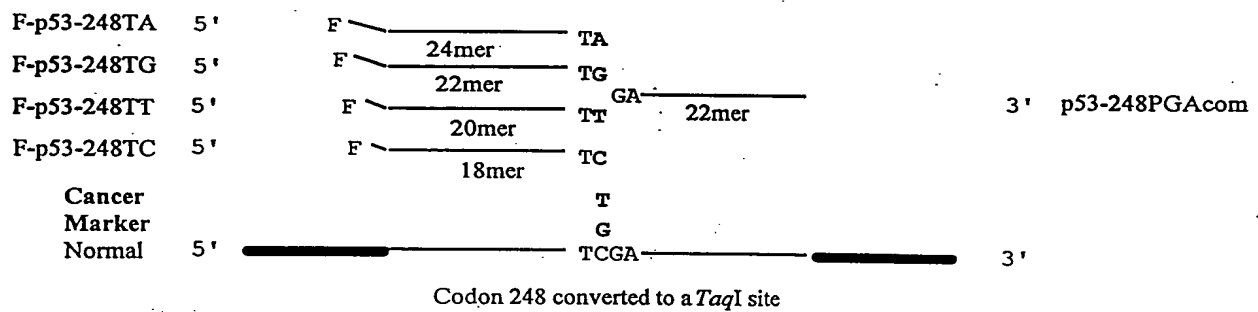


FIGURE 10

A

Primers

Ztop

CTT GGA CGA GTT CAT ACG C

p53zip248short

CTT GGA CGA GTT CAT ACG CGT TCC TGC ATG GGC GGC ATG A

p53-248short

GT TCC TCC ATG GGC GGC A→*pol*

|| ||| ||| ||| ||| ||| ||| |||

p53 exon 7

PCR product

(MK not shown)

p53-248shortR

p53zip248shortR

Zbot

*Msp*I (CCGG)

codon 248



3'...CA AGG AGC TAC CCG CCG TAC TTG GGC TCC GGG TAG GAG TGG TAG TAG TGT... 5' (-)

5'...GT TCC TCC ATG GGC GGC ATG AAC GGC AGG CCC ATC CTC ACC ATC ATC ACA... 3' (+)

pol-GG TAG GAG TGG TAG TAG TG

C GGG TAG GAG TGG TAG TAG TGC ACC GCT GGG TCA AAC C

C ACC GCT GGG TCA AAC C

B

Primers

Ztop

CTT GGA CGA GTT CAT ACG C

p53zip248T

CTT GGA CGA GTT CAT ACG CGT TCC TGC ATG GGC GGC ATG AAT

p53Taq248T

GT TCC TCC ATG GGC GGC ATG AAT

p53Taq248Q₆

TTCT TCC TCC ATG GGC GGC ATG AAQ₆→*pol*

|| ||| ||| ||| ||| ||| ||| |||

50-bp synthetic duplex DNA, or

PCR product

p53Taq248Q₆R

3'CA AGG AGC TAC CCG CCG TAC TTG GGC TCC GGG TAG GAG TGG TAG TAG TGT 5' (-)

5'GT TCC TCC ATG GGC GGC ATG AAC GGC AGG CCC ATC CTC ACC ATC ATC ACA 3' (+)

: ||| ||| ||| ||| ||| ||| ||| |||

pol-Q₆TCC GGG TAG GAG TGG TAG TAG TCTT

T TCC GGG TAG GAG TGG TAG TAG TG

T TCC GGG TAG GAG TGG TAG TAG TGC ACC GCT GGG TCA AAC C

C ACC GCT GGG TCA AAC C

C

LDR Primers

p53LDR248FTCL

p53LDR248FCA

p53LDR248FCG

p53LDR248FCT

p53LDR248FCC

Discrimination

Common

F-AAAAAAA GC ATG GGC GGC ATG AAT C

F-AAAAAA GC ATG GGC GGC ATG AAC A

F-AAAA GC ATG GGC GGC ATG AAC G

F-AA GC ATG GGC GGC ATG AAC T

F- GC ATG GGC GGC ATG AAC C *7'-ligase*

p53LDR248PGG

GG AGG CCC ATC CTC ACC ATC AT-block

conversion products

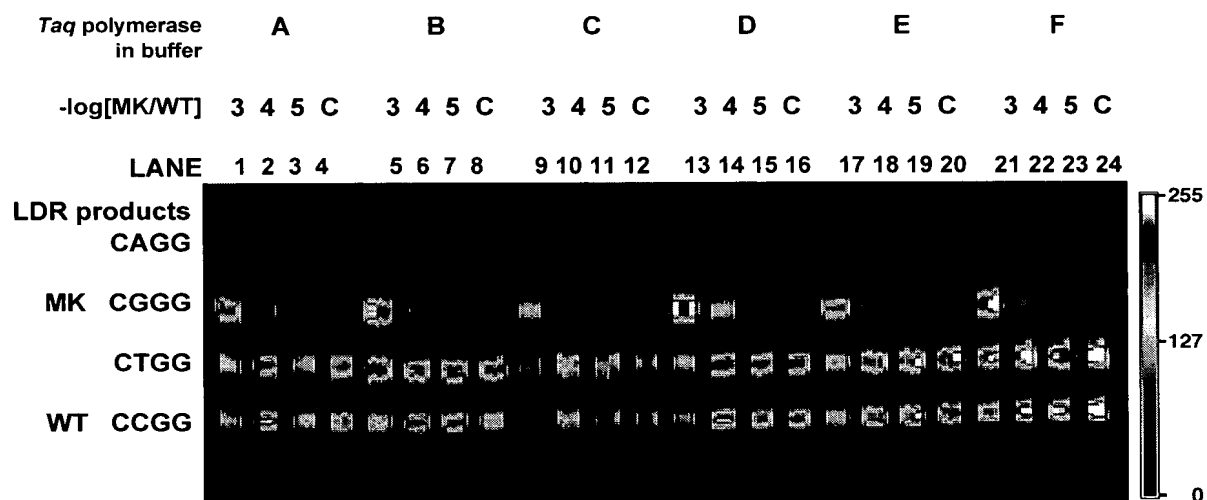
3' (- strand)

... GTA TGC GCA AGG ACG TAC CCG CCG TAC TTG NGG TCC GGG TAG GAG TGG TAG TAG TGA ACC...

5

FIGURE 11

A



B

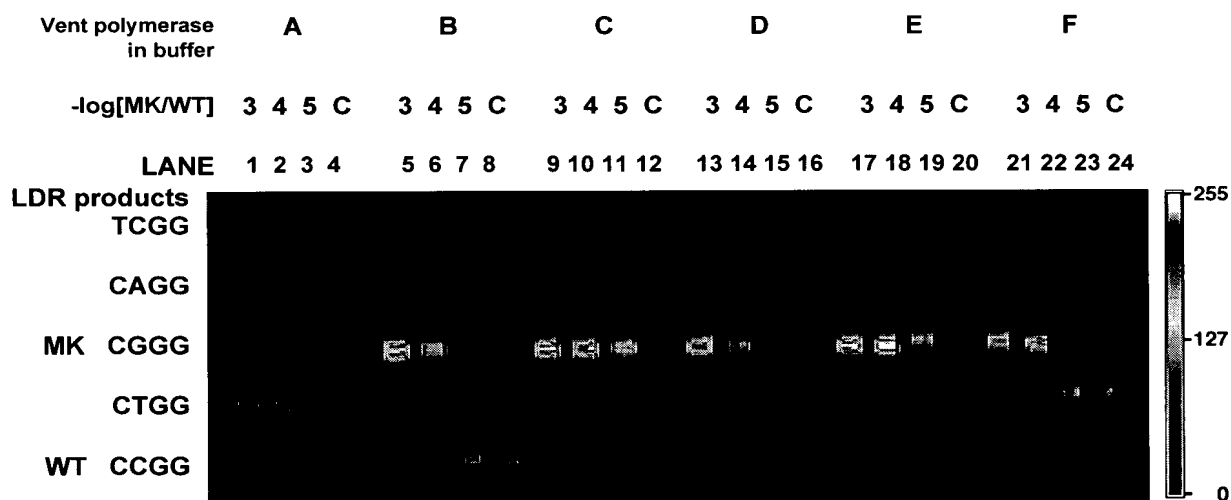


FIGURE 12

002120-11082560

Conversion	C:G				Q ₆ :G				T:G			
-log[MK/WT]	3	4	5	C	3	4	5	C	3	4	5	C
LANE	1	2	3	4	5	6	7	8	9	10	11	12

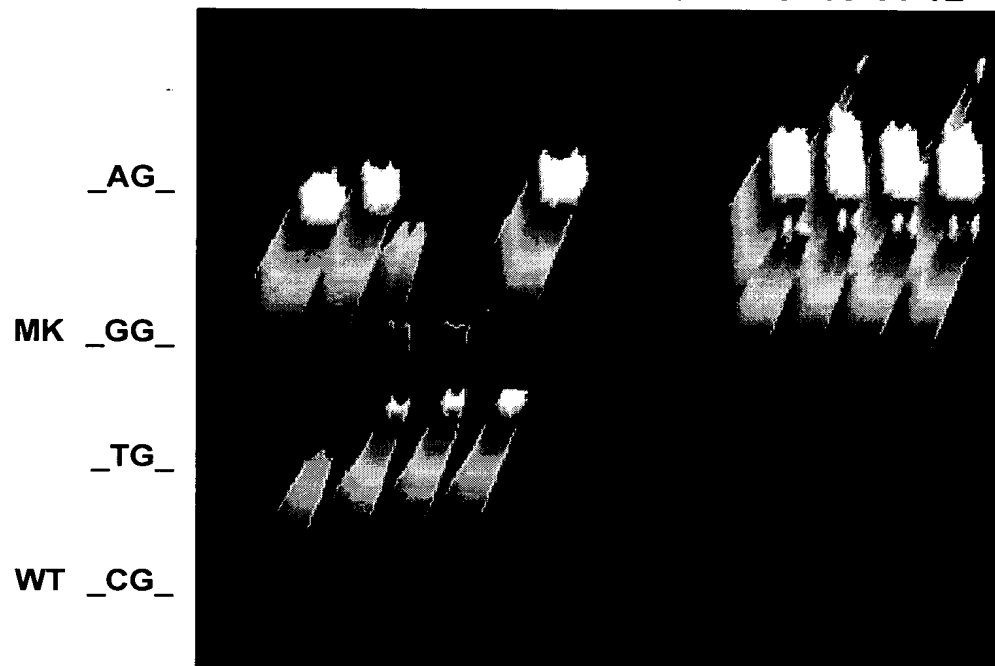


FIGURE 13